Analyses of sex and individual differences in vocalizations of Australasian gannets using a dynamic time warping algorithm

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(Received 6 July 2011; revised 22 May 2012; accepted 21 June 2012)

The study of the evolution of sexual differences in behavioral and morphological displays requires analyses of the extent of sexual dimorphism across various sensory modalities. In the seabird family Sulidae, boobies show dramatic sexual dimorphism in their vocalizations, and gannet calls have also been suggested to be dimorphic to human observers. This study aimed to evaluate the presence of sexually dimorphic calls in the Australasian gannet (*Morus serrator*) through the first comprehensive description of its vocalizations recorded at two localities; Cape Kidnappers, where individuals were banded and sexed from DNA samples, and at the Muriwai gannetry, both on the North Island of New Zealand. Calls were first inspected using basic bioacoustic features to establish a library of call element types for general reference. Extensive multivariate tests, based on a dynamic time warping algorithm, subsequently revealed that no sexual differences could be detected in Australasian gannet calls. The analyses, however, indicated extensive and consistent vocal variation between individuals, particularly so in female gannets, which may serve to signal individual identity to conspecifics. This study generates predictions to identify whether differences in Australasian gannet vocalizations play perceptual and functional roles in the breeding and social biology of this long-lived biparental seabird species. © *2012 Acoustical Society of America*.
[http://dx.doi.org/10.1121/1.4734237]

PACS number(s): 43.80.Jz, 43.80.Ka, 43.80.Lb, 43.80.Ev [JF] Pages: 1189–1198

I. INTRODUCTION

Considerable evidence regarding the extent and function of acoustic diversity in animal vocalizations comes from colonial species (Beecher, 1998; Beer, 1970; Stoddard, 1996). As the range of the perceptually discriminable properties of acoustic emissions may far exceed those of visual cues in a crowded colony, many seabirds appear to rely on vocal cues to recognize conspecifics, mates, and neighbors (Bretagnolle *et al.*, 1998). The breeding biology of seabird species in the family Sulidae (gannets and boobies), typically involves both a crowded colony environment and moderate-to-extensive physical similarity of adult individuals in size and coloration (Nelson, 1978); these traits predict a potentially critical biological context for a vocal-cue based social recognition system. For example, the Australasian gannet (Takapu in Maori; *Morus serrator*) is a large and predominantly white seabird with a buff yellow head and hind neck. These birds breed in dense colonies situated on sea-girt flat rocks in Australia and New Zealand (Nelson, 1978). Australasian gannets show a high degree of sexual monomorphism in size and human-perceived feather coloration, compared with related booby species, and there are no suggestions of differences in size or skeletal traits between adult females and males of this species (Daniel *et al.*, 2007). In contrast, subtle sex differences in the avian-perceivable wavelengths of light (including UV) of neck and back plumage coloration and in the proportion of black-to-white tail feathers may exist between females and males in this species (Ismar, 2010). As such, Australasian gannets, similarly to other sexually monomorphic species, are hypothesized to rely heavily on long-distance cues, including vocal signals, for sexual recognition (Taoka and Okumura, 1990), contributing to the evolution of sexually dimorphic vocal patterns.

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Sexual dimorphism is pervasive in many different avian taxa and can usually be explained as a consequence of sexual selection, owing to male competition and female choice (Darwin, 1871; Andersson, 1994). Sexual dimorphism may involve differences in size, plumage color, vocalizations, and/or behavioral repertoires (Owens and Hartley, 1998). In birds, sex differences are usually related to specific mating systems and patterns of parental care (Dunn *et al*., 2001). Surprisingly, in seabirds, sexual dimorphism appears to be decoupled from the strength of sexual selection, as revealed by the paucity of extra-pair parentage in a highly sexually dichromatic taxon (Cohen and Dearborn, 2004). Therefore, new research is required to establish and quantify the sensory modality and the biological extent of sex dimorphism across diverse seabird species.

In addition to its theoretical implications, human-perceived sexual monomorphism of size and color makes sexing seabird individuals unfeasible at field sites, and necessitates molecular sexing for accuracy (Griffiths *et al*., 1998; Daniel *et al*., 2007). However, some visually monomorphic seabird taxa produce notable differences in their sex-specific vocalizations. For example, in the blue petrel, *Halobaena caerulea*, calls can be used as indicators of sex and as predictors of intrasexual quality of breeding productivity (Genevois and Bretagnolle, 1994). Whereas most booby species (*Sula spp*.) show sex differences in integument colors and size, as well as in calls, the morphological aspects of sex differences are not at all remarkable to the human observer in the three closely related gannet species (*Morus spp*.) (Nelson, 1978; Daniel *et al*., 2007; Matthews *et al*., 2008; Ismar, 2010). This diversity of sexual dimorphism makes the Sulid family a case of particular interest in the study of visual versus acoustic sex differences. Specifically, Nelson (1978) mentioned anecdotal evidence of acoustic sex differences in only two of the three gannet species, in that the Cape gannet (*M. capensis*) and the Australasian gannet were claimed to show sex differences in the frequency of their calls which are audible to the human ear. In contrast, such sex differences were not investigated in the Atlantic (Northern) gannet (*M. bassanus*) (Nelson, 1978), despite several experimental bioacoustic studies on individual differences (White and White, 1970; White *et al*., 1970; White, 1971). Although Australasian gannets display extensive behavioral sexual dimorphism, especially related to breeding philopatry (Ismar *et al*., 2010a) and nesting strategies (Matthews *et al*., 2008), up until now, there has been no quantitative evidence for sex differences of vocalizations in this species.

The Australasian gannet emits three types of call, previously described by Nelson (1978) and Warham (1958): while taking off (skypointing call), while landing (shout call) and while defending its territory (alarm call), suggesting several distinct functions for the calls. The landing calls of the Atlantic gannet were previously studied (White and White, 1970; White *et al*., 1970; White, 1971) and these studies yielded acoustic and behavioral evidence for individual recognition. White and White (1970) suggested that a bird sitting on the nest may use the calls for identifying approaching birds. Individual recognition is typically associated with some other form of categorical recognition (Irwin and Price, 1999), such as species or sex recognition, and may be important in distinguishing a mate, sibling, offspring, cooperator, or rival (Tibbetts and Dale, 2007). Vocal individuality is also suggested to function in conspecific recognition for territorial defense and mate attraction (Beecher, 1998; Stoddard, 1996). Individual recognition in gannets could be of particular importance in recognition of territorial neighbors, incubating or brooding mates, and offspring, within the tightly packed colonial nesting environment.

Traditionally, individual recognition in birds has been tested through playback experiments (White and White, 1970). Advances in automating the otherwise time-consuming methods for bioacoustics (White and White, 1970; Anderson *et al*., 2009) are making possible the preliminary investigation of individual signatures in the features of the calls through the computational analyses of large amounts of acoustic data (Delport *et al*., 2002; Fox *et al*., 2008; Grava *et al*., 2008; Hoodless *et al*., 2008). In our study, we aim to provide the first quantitative tests of both vocal individuality and sexual dimorphism in the Australasian gannet using our extensive database of recorded calls. This database was first subdivided into sequences of sound emissions, termed here: "call elements," and defined as any continuous production of sound (Fig. 1) by visual inspection of spectrograms and manual extraction. We then employed an advanced multivariate bioacoustic analytical technique, adapted specifically for the comparison of different types and classes of avian vocalizations: dynamic time warping algorithm (Ranjard and Ross, 2008). Call elements were represented by a set of sound features which were used for univariate statistical descriptions. The central aim of our study was thus to describe the extent of variation in call elements across different biological predictors (individual identity and sex) using a spectrogram-analysis approach based on the time warping algorithm (Anderson *et al*., 1996; Brown and Miller, 2007; Ranjard and Ross, 2008; Ranjard *et al*., 2010). Using this multidimensional bioacoustic method, we assessed whether the resulting call element spectrograms of Australasian gannets were statistically different among *individuals,* and between the two *sexes*.

II. METHODS

A. Study sites and recordings

Vocalizing Australasian gannets were recorded at two study sites based around breeding colonies of the species. Individuals were studied at the Cape Kidnappers gannetry (39° 38′ S, 177° 05′ E), the largest mainland gannet colony in New Zealand (Wodzicki and McMeekan, 1947), situated 20 km south east of Napier. This site has been the focus of intensive study (e.g., Matthews *et al*., 2008; Ismar *et al*., 2010a; Ismar *et al*., 2010b; Ismar *et al*., 2011), including the collection of molecular sex data for metal sight-banded individuals from DNA samples (Daniel *et al*., 2007). The second study site was the Muriwai gannetry (36° 49′ S, 174° 25′ E), which is situated approximately 42 km northwest of Auckland City.

Audio recordings were obtained in 2006 and 2007 with each recording season conducted by a different observer. At

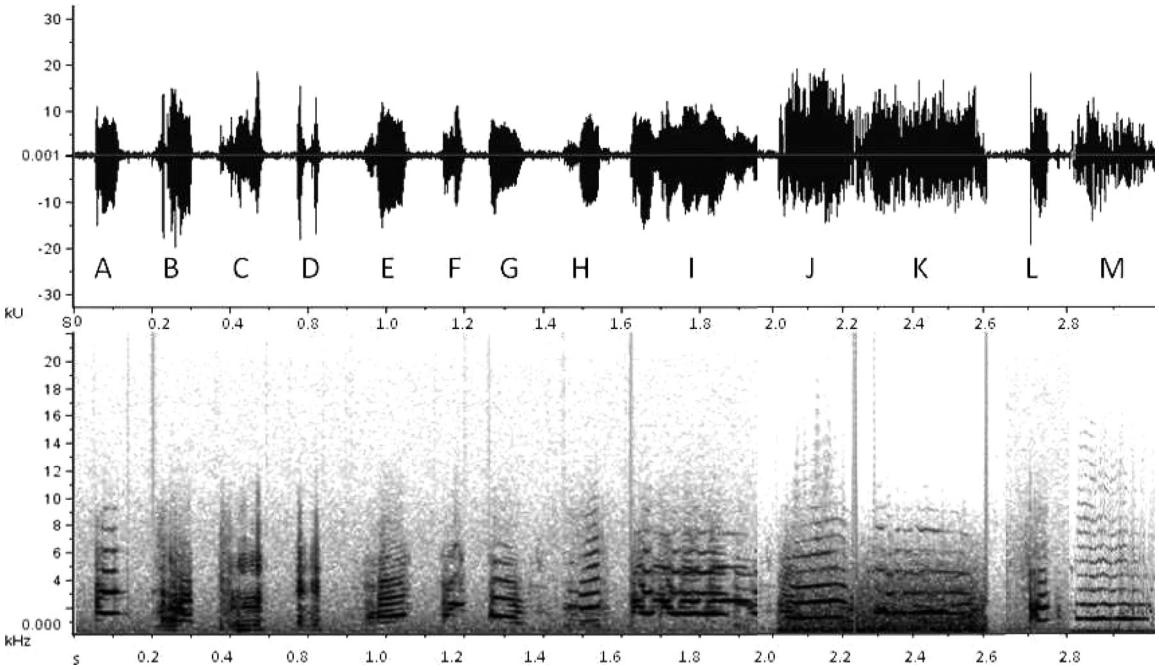

FIG. 1. Representative waveforms and spectrograms showing the library of the 13 call elements present in the Australasian gannet repertoire. The spectrograms were created using FFTs with 50% overlap and a frame size of 512 samples, resulting in 86.1 Hz frequency resolution and 5.80 ms time resolution, in RAVEN 1.2.0 (Cornell Laboratory of Ornithology).

both locations, the recordings were taken as close to the birds as these individuals would allow without moving away, to avoid the attenuation of harmonics with distance. Recordings of 23 different individuals chosen opportunistically amongst the colony's vocalizing gannets were made on three consecutive days (31 October–2 November 2006) at the Plateau, Black Reef, and Saddle subcolonies of the Cape Kidnappers gannetry. These recordings were made using a Fostex FR-2 Field Recorder with an Audio Technica Shotgun Microphone housed in a Rycote wind-kit (frequency response 20 Hz–20 kHz: +/− 1 dB). Sound files were then digitized at 48 kHz with 24 bit resolution. Unbanded individuals that were recorded in 2006 were captured, banded, and blood-sampled immediately following their recording so as to assign sex based on molecular markers in our laboratory (Daniel *et al.*, 2007). Opportunistic recordings of 29 additional previously banded and already known-sex individuals were also made in 2007 (3–6 September 2007) coincidentally with nest checks and taking audio notes on behavioral aspects of sex differences at the Plateau colony at Cape Kidnappers (Matthews *et al.*, 2008). These recordings were made using a Sony Dictaphone TCM 210 DV (frequency response in 200 Hz–6.3 kHz: ± 2.5 dB), using the normal speed (not the double recording time option), onto high fidelity cassette tapes to be digitized subsequently at 48 kHz with 24 bit resolution.

A further 43 individuals were recorded at the Atlantic and Southern cliff colonies at Muriwai gannetry, during two consecutive weeks (19–26 August 2007). Because gannets at this site are almost all unbanded and cannot be targeted for capture and genetic samples due to continuous tourist access to the site (and the associated limits on our research permits), these recordings were only used in the analysis of individual vocal differences. Every effort was made to record different gannets at this location by focusing on individuals attending different nest sites to avoid pseudoreplication. The Muriwai recordings were recorded onto a Marantz portable high resolution digital audio recorder (PMD 671) as 48 kHz with 24 bit resolution wave files using a handheld Sennheiser microphone (model K6 ME 66; frequency response in 40 Hz–20 kHz: ± 2.5 dB) (Table I).

The seasonal spread of the recording dates from Cape Kidnappers and Muriwai in 2007 was comparable as they were made at the same point of the breeding cycle (i.e., early pair formation in late August and early September 2007) (Tremain *et al.*, 2008). The set of recordings from 2006 at Cape Kidnappers, in turn, provided a contrast as they were made at a different point in the breeding cycle (i.e., late incubation and hatching, October to November 2006), but we also note that these recordings came from a different year of breeding. Due to the heterogeneous nature of our database (year, breeding stage, recording equipment, and personnel), we limited our study of acoustic variation in Australasian gannets to analyses of differences between individuals and between the sexes, recorded at the same site, breeding stage, and year (and, thus, all data collected by the same person and equipment); this was done to avoid biological and technical confounds owing to differences in recording equipment, time of year, and researcher behavior.

B. Acoustic measurements

All recordings, irrespective of initial method and media of data collection, were originally collected or digitized and saved as .AIFF files (with 44.1 kHz sampling rate and 16 bit precision) and visualized using RAVEN v1.2.0 (Cornell Laboratory of Ornithology, Bioacoustics Research Program, Ithaca, NY). A waveform view was first made from each

TABLE I. Source of origin of the 1952 call elements extracted from 71 calls of 47 individuals of the Australasian gannet at two North Island breeding colonies in New Zealand. Total sample sizes differ from the numbers of females and males because in some cases the sex of the recorded bird was unknown.

		Colony	
		Cape Kidnappers	Muriwai
Breeding stage	Pair formation	6 females: 150 elements	Sex unknown
		8 males: 333 elements	Sex unknown
		Total: 15 individuals, 507 elements	Total: 14 individuals, 479 notes
	Late incubation	6 females: 300 elements	None
		9 males: 579 elements	None
		Total: 18 individuals, 966 elements	None

sound file and corresponding spectrograms were generated using fast Fourier transform (FFT), with the RAVEN software's settings an FFT size of 512 samples, the Hann windowing function, and 50% overlap between frames resulting in a frequency resolution of 86.1 Hz and a time resolution of 5.80 ms. No filter was used in these analyses.

All recordings were examined visually and a call element library was constructed to characterize each different vocalization produced. In total, calls recorded from 47 individuals ($n = 15$ gannets in September 2007, 18 in October/November 2006, and 14 in August 2007) were analyzed for the library construction (Table I). The recordings were first categorized into call elements, or notes, defined as any continuous production of sound (Fig. 1). These were arbitrarily called by letters to distinguish one element from another. For each recording, every separate call element was characterized by measuring a predefined set of features. These included: highest frequency, lowest frequency, call element duration, the number of harmonics of the call element, the frequency at which maximum amplitude occurs, frequency range, and the position of the dominant harmonic.

Following the acoustic analyses of White *et al.* (1970) of Atlantic gannet calls, it was *a priori* decided that aspects of call amplitude were also important parameters to quantify. However, amplitude is affected by the distance at which a recording is made and, therefore, only the durations of amplitude increase (onset) and decrease (decay) were measured, as these characteristics would not be affected by the variable distance and environmental conditions during our sound recording attempts. Using these measurements, gannet vocalizations were then described to produce a library of call elements common to the species. The detailed acoustic and statistical features of each call element are described in Table II.

C. Statistical comparisons

To validate visual assessment of gannet call elements quantitatively, a Mantel test (10 000 random replicates, Fig. 2) was performed on the different call elements of the call element library constructed based on visual inspection of the spectrograms.

We also used a multivariate approach to extract individual, sex specific, seasonal, and geographic variation within and between Australasian gannet vocalization call elements. For this analysis, we used the sound comparison approach developed and described in detail by Ranjard and Ross (2008) for avian bioacoustic analyses, based on cepstrum coefficients. These metrics are commonly used in processing human speech to encode spectrograms and represent the balance of energy between the different frequencies of the spectrum (Davis and Mermelstein, 1980). Even if not originally designed for non-human bioacoustic analyses, the approach has been shown to be effective for the study of other animal, including avian, vocalizations (Kogan and Margoliash, 1998; Ranjard and Ross, 2008; Ranjard *et al.*, 2010; Brown and Smaragdis, 2009).

For our analyses of Australasian gannet vocalizations, a sequence of mel-frequency cepstrum coefficients was extracted from each element using the Hidden Markov Model Toolkit (Young *et al.*, 2006). The first of the 26 filterbank channels started at 1 kHz and the last one terminated at 22.05 kHz, resulting in a shifted mel-scale. Under a sampling frequency of 44.1 kHz, a Hamming window of 128 samples with 50% overlap was used for computing the spectra and the signal had first-order pre-emphasis applied using a coefficient of 0.97. Twelve coefficients were calculated and the 0th cepstral parameter was used as the energy component, resulting in a total of 13 coefficients. Two frames before and two frames after the current one were used to estimate the first and second-order temporal derivatives leading to a total number of 39 coefficients. Liftering was applied to rescale the cepstrum coefficients so that they had similar magnitudes. For this purpose, a coefficient of 22 was used in the formula defined by Young *et al.* (2006) (Fig. 2).

A dynamic time warping algorithm was used to compare the call element cepstrum coefficients sequences and detect similarities between the call element spectrograms, while also taking into account the differences in the phase of these sequences. An optimal alignment of the two encoded spectrograms was computed and the distance was defined as the number of operations required to obtain this alignment. Five operations were allowed: substitution, insertion, deletion, compression, and expansion. Therefore, this distance measure was dependent on both the frequency and the time parameters of the call elements (for details, see Ranjard and Ross, 2008 and Ranjard *et al.*, 2010). Using this algorithm, a call element pair-wise distance matrix (1952×1952) was constructed which quantifies the acoustic similarities between elements independently from the library. This call element pair-wise distance matrix was used to ask if the call elements visually classified into the same type in the library are significantly less distant to each other than to the notes

TABLE II. Mean acoustic features of the 13 call elements identified in the Australasian gannet call repertoire library across all recordings. Lowest frequency, highest frequency, frequency range, and the frequency at which maximum amplitude occurs were calculated for the dominant harmonic.

		A	B	C	D	E	F	G	H	I	J	K	L	M
Number of harmonics	Mean	7.3	18	14.89	11.58	11.77	8.44	10.34	6	13.67	13	6	6.71	8
	Standard error	0.16	0.72	1.38	0.25	0.6	0.31	0.97	1.05	5.7			0.35	1.11
Position of the dominant harmonic	Mean	1.55	1.98	1.78	2.06	1.25	1.43	1.42	1.4	2	2	1	1.5	1.09
Onset (s)	Mean	0.03	0.03	0.03	0.02	0.04	0.03	0.03	0.03	0.05	0.07	0.05	0.02	0.09
Decay (s)	Mean	0.03	0.02	0.02	0.02	0.17	0.02	0.05	0.02	0.05	0.1	0.05	0.02	0.1
	Standard error	0.0002	0.0001	0.0001	0.0001	0.14	0.0001	0.0001	0.0001	0.01			0.0001	0.02
Lowest frequency (Hz)	Mean	1590.05	892.65	891.56	1199.52	1073.87	1344.92	1160.94	1384.1	1145.93	2267	1425	1323.88	547.03
	Standard error	31.32	74.62	156.07	27.95	46.58	42.16	56.16	59.71	71.16			46.17	108.18
Highest frequency (Hz)	Mean	2043.66	1326.84	1318.62	1686.27	1564.49	1877.98	1729.3	2118.78	1875.93	2721	1749	1817.7	1002.29
	Standard error	31.41	79.5	175.11	29.54	48.69	43.28	58.96	115.98	216.1			48.91	126.59
Frequency range (Hz)	Mean	453.61	434.2	427.05	486.76	490.62	533.06	568.35	734.68	730	453.4	323.9	493.82	455.23
Call element duration (s)	Mean	0.06	0.08	0.08	0.06	0.07	0.05	0.1	0.06	0.22	0.17	0.25	0.05	0.48
Frequency at which maximum amplitude occurs (Hz)	Mean	1833.19	1133.33	1150.14	1468.43	1374.93	1625.65	1497.51	1722.66	1665.23	2531	1688	1585.87	806.51
	Standard error	30.86	79.04	168.17	28.82	47.24	41.66	56.94	76.9	207.29			46.51	116.46

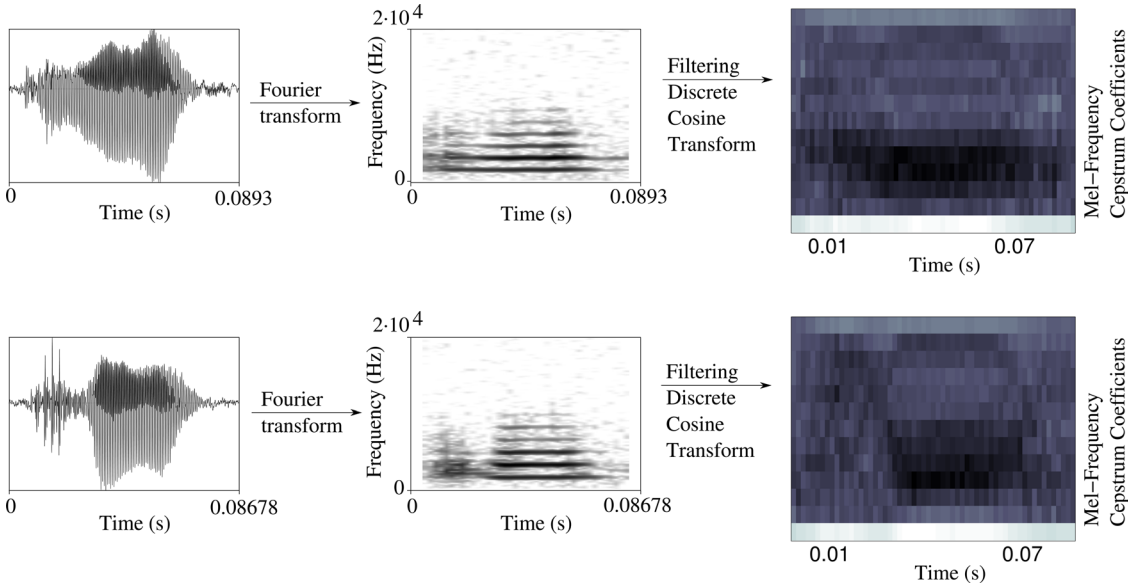

FIG. 2. (Color online) Schematic representation of the extraction of mel-frequency coefficients from two call elements. First, the windowed Fourier transform is calculated. Second, the power of the spectrum is filtered according to the mel-scale. Finally, the discrete cosine transform of the log of the magnitude of each mel-frequency is computed. Each call element is then encoded as a matrix with mel-frequency cepstrum coefficients magnitude through time (following Ranjard and Ross, 2008).

belonging to the other call element types. Sokal and Rohlf (1995) proposed the use of Mantel tests for analyzing the correlation of distance and groups in a data set. Such tests were performed to assess the correlation between the call element pair-wise distance matrix and a binary matrix indicating, for each pair of call elements, if they belong to the same call element type as determined by our library classification. Therefore, a significant correlation would indicate a difference in the call elements belonging to different types in the library.

Using the same call element pair-wise distance matrix, additional Mantel tests were used to investigate if the distances were significantly correlated with individual and sex. This approach permitted us to evaluate the following questions.

(1) Were the call elements extracted from the recordings of the same *individual* more similar to each other than among *individuals*?
(2) Were the call elements extracted from the calls of individuals of the *same sex* more similar to each other than to notes produced by individuals of the *different sexes*?

For each of these two tests, a specific call element pair-wise identity binary matrix was built. These binary matrices indicated which call elements were identical in regards to the factor *individual* and the factor *sex*. For each factor, the binary matrix indicated if the two call elements had the same (distance 0) or different (distance 1) values. For example, using the *sex* factor identity matrix, if two call elements were recorded from two birds of the same sex, whether from different birds or from the same bird, a distance of 0 was given to this call element pair. Similarly, for the *individual* factor, the pair-wise distance was 0 if the call elements were extracted from calls of the same individual and 1 otherwise. These two identity matrices can be seen as defining the groups in the call element dataset. For instance, the *sex* identity matrix, groups the call elements into two sets with elements produced by males in one group and elements produced by females in the other. In a similar way, the call elements were grouped according to individual. Because the sexes were not evenly sampled (Table I), the sizes of the identity matrices differ (Table II). The confidence intervals for each correlation coefficient were then calculated according to the method described in Manly (1997).

In this study of Australasian gannet vocalizations, the sex and individual factors are not independent; the call elements of the same individual necessarily belong to the same sex. Therefore, when testing a given factor, the other factor needed to be fixed. To fix the *individual* factor, an average call element was calculated for each bird. Given a particular individual for which a list L containing N call elements have been recorded, the average call element sequence was obtained using the following recursive method.

(a) Calculate the acoustic pair-wise distance matrix (D) between the encoded spectrograms of the call elements of L.
(b) Find the indices a and b of the two most distant call elements, and compute the warping path between them using the algorithm described in Ranjard and Ross (2008) (Fig. 3).
(c) Compute the average sequence from this warping path.
(d) Replace the encoded spectrograms of call elements a and b by their average sequence in L and recalculate the pair-wise distance matrix, the size of L, as well as the rank of D decrease by 1 at this stage.
(e) *If* the number of call elements of L is greater than 1, *then* go back to the step (a) *otherwise* finish the procedure.

Once this procedure was completed, a single sequence was returned which consisted of the average of all the encoded call elements recorded for this particular individual.

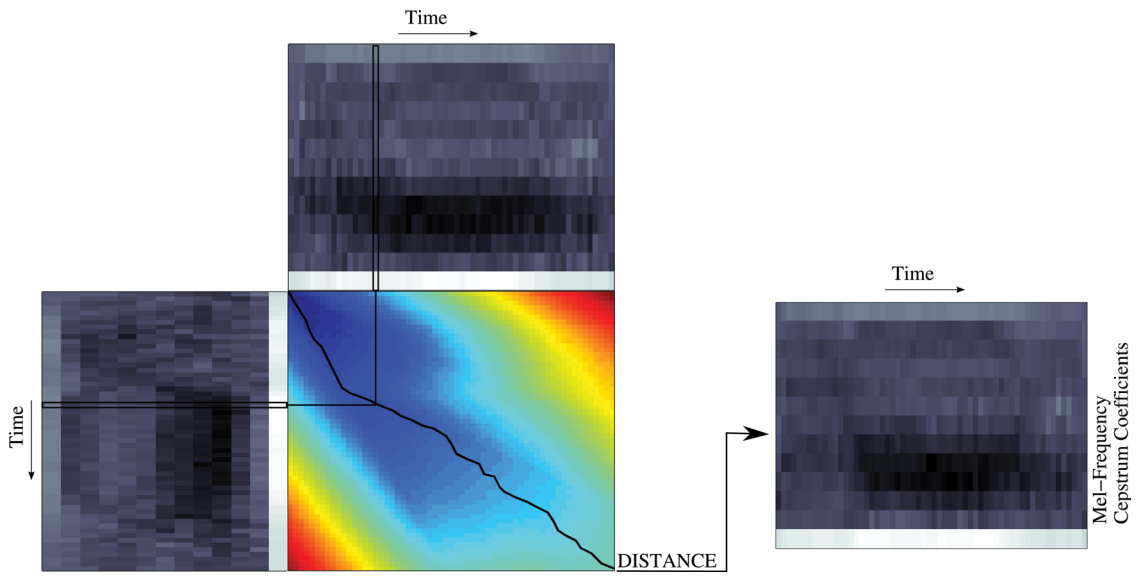

FIG. 3. (Color online) Alignment of two encoded Australasian gannet call elements of Fig. 2. A dynamic programming algorithm is used to find the best time mapping between the two mel-frequency cepstrum coefficient matrices (dynamic time warping). This mapping is represented by a path (black line) in a cost matrix that takes into account the similarity between the coefficient vectors through time. A cost is associated to the different possible time alignments and the minimal cost constitutes the distance score between the two call elements. Moreover, an average sequence can be generated by tracing back the alignment path (black line), and averaging the coefficient vectors (black rectangles) of the two call elements at each time step [see Ranjard and Ross (2008) for the analytical description of the method].

The 47 individuals' average call elements were aligned using the same dynamic time warping algorithm which resulted in a 47 by 47 distance matrix. Consequently, this matrix indicated the acoustic distance for each pair of birds based on their average call elements. An additional *individual* identity matrix was built for the sex factor. The correlation between these identity matrices with the acoustic distance matrix was tested using the Mantel test. This framework resulted in eight different statistical tests. Following a conservative statistical approach, we applied a Bonferroni correction (Abdi, 2007), with the significance level was set at $\alpha = 0.004$ (0.05/14).

III. RESULTS

A. A library of Australasian gannet call elements

The survey of Australasian gannet vocalizations from both breeding sites (Table I) revealed a vocal repertoire of 13 acoustically distinct call elements. The first half of the call elements identified (Fig. 1) are typically used by individuals whilst calling on the nest or when interacting with a landed partner. The second half of the call elements described were used while in flight or taking off. Call element J was recorded solely when approached by an observer, suggesting that it may be an alarm or distress call. The Mantel test, performed on the different call elements of this library, indicated that the call elements classed by the human observer as belonging to the same call element type, were significantly more similar to each other than to other call elements ($r = 0.04$, confidence interval 99%: [0.03, 0.05], $p = 0.0001$).

B. Factor analyses of acoustic differences

We identified statistically significant differences for the factor *individual* but not for the factor *sex*. Results of the eight tests aimed to detect correlations between the tested factors and their acoustic distance are summarized in Table III. Two tests resulted in significant positive correlations which explained moderate levels of variability in the data ($r > 0.10$).

In detail, a significant positive correlation was revealed between individual identity and the acoustic distance of call elements in the tests involving female elements at either breeding season stages included in our sampling protocol (Fig. 3). This implies that the call elements produced by a particular female individual are more similar to each other than to the call elements of other females. In contrast, no significant correlation was detected between the call element distance of males during pair formation in Cape Kidnappers and the *individual* identity matrix. This suggests that during the pair formation stage of the breeding season, a particular male does not produce call elements that are statistically different from those of other males, whereas females do. The male call elements recorded later in the breeding season yielded a significant positive correlation but the corresponding coefficient of correlation was low (0.03). Overall, these results imply acoustically detectable consistency in individual identity of call elements produced by Australasian gannets across both sexes.

Initially, the *sex* and acoustic distance matrices showed evidence of significant correlation, at the late stage of the breeding season, when performed with the original set of call elements. However, these tests involved several call elements for each individual which means that in these two cases, the within *individual* variation was contained in the within *sex* variation. In other words, the distances between the call elements of the same individual, which have been shown to be smaller than expected by chance for both females and males at this later breeding stage (see above), are included in the distances between call elements of individuals of the same sex. To test the factor *sex per se*, it was therefore necessary to remove the effect of the factor *individual*. By using only a single average sequence of call elements per individual and, thus, fixing the factor *individual*, the results of factor *sex* were no longer statistically significant (Table III with factor *individual* fixed). Therefore, the effect of the factor *individual* is responsible for the correlation observed in the first two tests. This implies that the amount of acoustic variation among the call elements produced by individuals of the same sex is of similar magnitude to the amount of variation found between call elements of the different sexes.

IV. DISCUSSION

A. Sources of variation in Australasian gannet calls

We obtained a data set of Australasian gannet vocalizations from diverse sources using different recording approaches. Accordingly, it was expected that owing to

TABLE III. Results for eight Mantel tests of correlation (10^4 replicates of random permutations). Tests significant at $\alpha < 0.004$ (Bonferroni correction for 0.05 significance level) are shown in bold and the 99% confidence intervals are indicated in square brackets. "Cape Kid." stands for Cape Kidnappers gannetry, "pair form." for pair formation, and "late inc." for late incubation breeding stage. The factor "individual" was fixed by computing a single averaged call element for each bird (see text).

Factor	Number of calls	Individual	Sex	Breeding stage	Colony	Correlation
	150		F	pair form.	Cape Kid.	**0.12 [0.09,0.15]**
Individual	300		F	late inc.	Cape Kid.	**0.12 [0.09,0.15]**
	333		M	pair form.	Cape Kid.	−0.01 [−0.05,0.03]
	579		M	late inc.	Cape Kid.	**0.03 [0.01,0.05]**
	483		F + M	pair form.	Cape Kid.	−0.05 [−0.10,0.01]
Sex	14	fixed	F + M	pair form.	Cape Kid.	0.04 [−0.26,0.24]
	879		F + M	late inc.	Cape Kid.	**0.09 [0.06,0.12]**
	15	fixed	F + M	late inc.	Cape Kid.	0.18 [−0.14,0.46]

technical, methodological, and biological variation of our recording sources, any bioacoustic analysis would reveal high levels of variation in the call structure of our subjects. This prediction was confirmed using both visual and univariate inspections (e.g., call element library) and detailed quantitative analyses (e.g., time warping algorithms and Mantel tests); critically, however, these effects might represent potential artifacts resulting from the disparate methods of selecting subjects, recording calls, and digitizing data files. To the contrary of this possibility, we have detected shared acoustic structure and similarity of gannet vocalizations within otherwise heterogeneous data sources (e.g., similar calls within the same stages of the breeding season, consistent sexual monomorphism of call structure, irrespective of recording site and time), despite calls having been recorded by various observers using different equipment at distant sites. In turn, our analyses also revealed acoustically different call structures from homogeneous data sources, recorded by an observer using identical equipment at the same site (e.g., individual differences in gannet calls at Cape Kidnappers, especially of females, both in the early or in the mid breeding season). Taken together, these patterns of variability imply a biological reality, rather than methodological artifacts, represented in our bioacoustic survey and analysis of New Zealand-breeding, adult Australasian gannet calls.

B. Vocal repertoire library

This study provides the first detailed description of the Australasian gannet vocal repertoire. Extensive analyses of vocalizations recorded from our sample of Australasian gannets demonstrate that these birds have a complex repertoire of call elements that are both consistent in structure and variable across different scales of biological levels. Some call elements were observed to coincide with certain behaviors; however, more focused studies observing and experimentally addressing the role of behavioral contexts on call element use-patterns in this species would be required in the future to confirm this (Douglas and Mennill, 2010), as it was not the focus of this study.

C. Factor-related differences in Australasian gannet vocalizations

1. Individual differences

The results of our study provide the first quantitative evidence of distinct individual differences within Australasian gannet vocalizations. This result parallels the conclusions, but not the methods, of White *et al*. (1970) who found individual differences in the calls of the congeneric Atlantic gannet. The acoustic approach used here was aimed at simultaneously testing multiple acoustic factors separating Australasian gannet calls, rather than detecting the individually consistent acoustic components of calls, in part because absolute amplitude measures were not available to us to parallel the detailed bioacoustic feature analysis of the calls reported by White *et al*. (1970).

Moreover, individual aspects of the calls were found only at the onset of call bouts of the Atlantic gannet (White and White, 1970). This could mean that only a subset of call elements within a calling bout would contain signatures of individuals. Krull and Hauber (2010) found evidence for a two voice system in the Australasian gannet, and considered whether this was important in generating variability for individual recognition in this species. The second voice was only found in calls made by gannets flying over the colony or in the first few call elements after landing, and so Krull and Hauber (2010) specifically suggested that double voicing would be required in the initial vocal recognition in these behavioral contexts, after which it is no longer needed.

Similarly, consistent differences in the multidimensional acoustic traits of different individuals' calls were detected in the Australasian gannet across our different sources of recordings, although these methods did not allow us to identify which acoustic traits were responsible for these differences between individuals. Also, it remains to be determined experimentally whether any of the acoustic differences in individual calls of Australasian gannets are indeed perceivable to evoke different physiological and behavioral responses in conspecific listeners, as was demonstrated experimentally by White *et al*. (1970) for adult males of the Atlantic gannet using playbacks.

Vocal individuality may also be important in some species for the recognition of parents by offspring (Beecher, 1998; Anderson *et al*., 2010). Accordingly, although White (1971) found that while some Atlantic gannet chicks had the ability to recognize their parent in a playback test, they did not behave as if they always did so. This may be because there is no particular advantage for vocal recognition between parents and young in Atlantic gannets as the typical clutch size is one egg, and the single chicks attended by the parents are initially not mobile and remain on the nest until several weeks after hatching (Nelson, 2002). In contrast, the Australasian gannets differ in that the chicks venture from the nest to the outskirts of the colony in the last few weeks before fledging and these young may, therefore, benefit from more consistent adult vocal signatures for parent-offspring recognition (Nelson, 1978).

Our findings show that vocalizations of female Australasian gannets are more distinct from those of other females, than are calls of males from those of other males. This is particularly interesting in view of the fact that territorial establishment (as in the Atlantic gannets, Nelson, 2002) and nest defense may benefit males more in this species (Matthews *et al*., 2008). Male-biased territorial aggression in gannets is consistent with reports from other seabird species (Paredes and Insley, 2010; Creelman and Storey, 1991; Fraser *et al*., 2002). In this scenario of behavioral sex-dimorphism, females with distinguishable calls would be selected for by being granted more ready access to their nest-site, and suffer less accidental aggression from their pair-bonded male mate due to mis-identification.

2. Sex differences

Previous observations by Nelson (1978, 2000) and Marchant *et al*. (1990) had suggested sex dimorphism in the human-perceived frequency of Australasian gannet calls,

with Nelson (1978) explicitly proposing that a male's call is higher pitched than the female's call. To the contrary of this prediction, we found no evidence for consistent sexual dimorphism in Australasian gannet calls.

Sex differences in vocalizations in some cases have been directly related to size differences between the sexes with typically the larger of the two sexes producing lower frequency calls (Farquhar, 1993). During a recent study of Australasian gannets (Daniel *et al.*, 2007) we found that males had on average greater bill length, depth and width, whilst females had larger tail length and body weight measurements; however, only male bill width measurements were found to be statistically greater relative to females, and even those were on average a minute 0.5 mm (<1% of the absolute size) different between the sexes. This does not coincide with the observation by Nelson (1978, 2000) and Marchant *et al.* (1990) that the male Australasian gannet call would be significantly higher than the females. If Farquhar (1993) is correct, and bill width in the Australasian gannet does have an effect on the frequency of their call, then males should emit a lower frequency call than females. A preliminary univariate analysis of size indicated no detectable statistical effect of bill or body size of banded birds on their respective recorded calls' acoustic parameters (Krull and Hauber, 2010).

The question, therefore, remains as to how, if not by vocal cues, an Australasian gannet determines the sex of a conspecific. Daniel *et al.* (2007) suggested sexual size monomorphism and human-perceived monochromatism amongst genetically sexed individuals. In contrast, behavioral correlates of sex specificity are increasingly detected amongst Australasian gannets, involving breeding site fidelity (Ismar *et al.*, 2010a) and prelaying nesting displays (Matthews *et al.*, 2008). Combining behavioral and visual sensory cues may be sufficient in Australasian gannets to identify opposite sex partners and same sex competitors around the nesting site.

V. CONCLUSIONS

The Australasian gannet has a qualitatively complex repertoire of call elements, which is described in detail for the first time in this report. Vocal diversity of individuals might contribute to this species' intricate mating displays (Matthews *et al.*, 2008), the critical role of pair formation and maintenance in promoting timely breeding and successfully fledging young within and between years (Ismar *et al.*, 2010a); we have thus demonstrated a need to assess the role of vocalizations in future work using acoustic playback experiments (Parker *et al.*, 2010) and in the analyses of potential multimodal sensory signals for social recognition in this species.

In relation to the specific hypotheses of this study, the Australasian gannet call was found to show individual differences in acoustic signatures, similarly to previous evidence on individually different vocalizations in the related Atlantic gannet (White and White, 1970). Contrary to predictions based on the empirical literature and theoretical considerations, we detected no sexual differences in calls from individuals whose sex was determined unambiguously using genetic markers (Daniel *et al.*, 2007). These results suggest a reliance on sensory and behavioral cues other than calls for initial mate selection and sex recognition in the Australasian gannet. Overall, this study illustrates the application of a powerful bioacoustic analytical approach technique for the study of sex differences (Ranjard and Ross, 2008; Ranjard *et al.*, 2010) and provides strong foundation for future acoustic playback experiments to understand the role of the vocal repertoire and the different biological scales of signal variability in Australasian gannet vocalizations.

ACKNOWLEDGMENTS

C.R.K. and L.R. have contributed equally to this research. Funding was provided by the University of Auckland and its Faculty of Science Research Committees, the New Zealand Marsden Fund, Education New Zealand, and the Auckland Museum. The study was conducted under required institutional and governmental research permits. For comments and help with the analyses, we are grateful to Howard Ross and for assistance and discussions we thank Grant Ballard, Don Dearborn, Andrea Gager, Craig Millar, Selina Patel, Brent Stephenson, landowners and staff of the Cape Kidnappers Reserve, the New Zealand Department of Conservation, many other colleagues, and the anonymous referees of the journal.

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